REMARKS

A check for the fee for a three month extension of time accompanies this response. Any additional fees that may be due in connection with filing this paper or with this application during its entire pendency may be charged to Deposit Account No. 50-1213. If a Petition for extension of time is required, this paper is to be considered such Petition, and any fee charged to Deposit Account No. 50-1213.

Claims 1-57, 59, 61-83 and 141-143 are pending in this application. Claims 58, 60 and 140 are canceled herein without prejudice or disclaimer. Applicant reserves the right to file divisional or continuation applications to any canceled claims or unclaimed subject matter disclosed in the specification. Claims 1, 50 and 65 are amended to correct minor typographical and grammatical errors and to more particularly point out the claimed subject matter. Basis for the amendments to Claim 1 may be found throughout the specification, which provides methods for delivery of large nucleic acid molecules. Particular basis may be found, for example, on page 2, line 10 to page 4, line 9. Basis for the amendment to claim 65 may be found in the specification, for example, at page 2, lines 17-20; page 23, lines 3-8 and at page 25, lines 22-25. Claim 59 is re-written as an independent claim incorporating the features of canceled Claim 58. Claim 59 further specifies that the size of the large nucleic acid molecule is at least 5 megabases; basis for this amendment may be found in the specification, for example, at page 9, line 30 to page 10, line 3, which defines large nucleic acid molecules as including nucleic acid molecules of at least about 5 megabases. Claim 141 is re-written as an independent claim incorporating the features of canceled Claim 140.

Claims 144-147 have been added. Basis for Claims 144-147 may be found, for example, at page 10, lines 4-7, which defines the size ranges of large nucleic acid molecules.

The amendment to the paragraph in the specification corrects a minor obvious typographical error.

A Supplemental Information Disclosure Statement also is filed on the same day herewith under separate cover.

THE REJECTION OF CLAIMS 1-83 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

A. The Enablement Rejection

Claims 1-83 are rejected under 35 U.S.C. § 112, first paragraph because it is alleged that the specification, while being enabling for a method of introducing a nucleic acid into a cell *in vitro*, does not reasonably provide enablement for *ex vivo* or *in vivo* gene transfer for gene therapy.

The Office Action alleges that because the specification provides no practical utility for *ex vivo* or *in vivo* gene transfer other than gene therapy, the disclosure, in order to be enabling, must teach a skilled artisan how to use the claimed method for the purpose of gene therapy.

It is further alleged that the state of the art in regards to *ex vivo* or *in vivo* gene therapy, at the time of filing of the instant application, was considered to be highly unpredictable, regardless of the mode of delivery. The Office Action cites a collection of references from 1995-2001 (listed and discussed below), which allegedly teach the unpredictability of gene therapy. The Office Action concludes that the alleged lack of demonstration of the *in vivo* efficacy of gene therapy in the specification, when coupled with the aforementioned unpredictability of gene therapy, suggests that the teachings of the instant application would not permit one of skill in the art to practice the methods without undue experimentation.

This rejection is respectfully traversed. It is respectfully submitted that this rejection has been rendered moot with respect to Claims 58 and 60, which are canceled herein.

Relevant law

To satisfy the enablement requirement of 35 U.S.C § 112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. Atlas Powder Co. v. E.I. DuPont de Nemours, 750 F.2d 1569, 224 USPQ 409 (1984). This requirement can be met by providing sufficient disclosure, either through illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything *within the scope* of a broad claim." In re Anderson, 176 USPQ 331, at 333 (CCPA 1973), emphasis in original. Rather, the requirements of § 112, first paragraph "can be fulfilled by the use of illustrative examples **or** by broad terminology." In re Marzocci et al., 469 USPQ 367 (CCPA 1971)(emphasis added).

Further, because "it is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species, it is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it." In re Grimme, Keil and Schmitz, 124 USPQ 449, 502 (CCPA 1960). Thus, there is no doubt that a patentee's invention may be broader than the particular embodiment shown in the specification. A patentee not only is entitled to narrow claims particularly directed to the preferred embodiment, but also to broad claims that define the invention without a reference to specific instrumentalities. Smith v. Snow, 294 U.S. 1, 11, 24 USPQ 26, 30 (1935).

Thus, there is no requirement for disclosure of every species within a genus. Applicant is entitled to claims are commensurate in scope not only with what applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed.

The inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the claimed invention. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988).

Analysis

1. **Application of the Above-noted Factors**

Scope of the claims

Claims 1-83 are directed to methods for introducing a nucleic acid molecule into a cell. Independent claim 1 is directed to a method of introducing a large nucleic acid molecule into a cell by exposing the cell to a delivery agent, exposing a large nucleic acid molecule to the delivery agent, and contacting the cell with the nucleic acid molecule. The steps can be performed in any order sequentially except if the delivery agent is energy it is not applied to the nucleic acid molecule or to the cell after contacting the cell with the nucleic acid molecule. Independent claim 34 is directed to a method of delivering a nucleic acid molecule to a cell where the cell is contacted with a delivery agent and ultrasound or electrical energy and then the cell is contacted with the nucleic acid molecule. Independent claim 48 is directed to a method of delivering a nucleic acid molecule into a cell in a subject by administering a delivery agent alone followed by application of ultrasound or electrical energy and then administering the nucleic acid molecule. Independent claim 59 is directed to a method for delivering a large nucleic acid molecule into a cell, that includes

contacting the nucleic acid molecule with a composition that comprises a cationic lipid composition of 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoroacetate (DOSPA) and dioleoylphosphatidylethanolamine (DOPE), where the nucleic molecule is at least 5 megabases in size; and then contacting the nucleic acid molecule with a cell, wherein steps (a) and (b) are performed simultaneously or sequentially. Independent claim 65 is directed to a method for delivering a nucleic acid molecule into a cell in a subject by mixing the nucleic acid molecule with a delivery agent, administering the mixture to the subject and applying ultrasound or electrical energy. Independent claim 73 is directed to a method of delivering a nucleic acid molecule into a cell in a subject by applying ultrasound or electrical energy to the subject followed by the application of a nucleic acid molecule and a delivery agent sequentially or as a single composition. Independent claim 80 is directed to a method for delivering a nucleic acid molecule by applying ultrasound or electrical energy to the subject and administering a nucleic acid molecule upon conclusion of the application of energy. Dependent claims further specify size ranges and types of nucleic acid molecules; methods *in vitro*, *ex vivo* and *in vivo*; types of delivery agents; treatments and parameters for using the delivery agents; the order in which the steps of the methods are practiced; and cell types.

Thus, all of the instant claims are directed to methods for delivering nucleic acid molecules into cells, not methods of gene therapy. The steps of the methods encompass how such delivery may be accomplished either *in vitro*, *ex vivo* or *in vivo*. The uses of nucleic acid delivery, including gene transfer for gene therapy, constitute only a subset of the embodiments that are within the scope of the subject matter as instantly claimed.

Level of Skill

The level of skill in this art is recognized to be high (see, *e.g.*, Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986)). The numerous articles

and patents made of record in this application address a highly skilled audience and further evidence the high level of skill in this art.

Teachings of the specification

The instant application is directed to methods for delivering a nucleic acid molecule into a cell. The specification teaches that these delivery methods have numerous utilities in medical, pharmaceutical, biological and technology-related research fields including production of proteins, generation of transgenic animals, uses with imaging methods for medical devices, mapping biological events *in vivo*, gene delivery optimization technology development, generation of transplantable organs and tissues and *in vivo* and *ex vivo* therapies. For example, on page 6, lines 3-9, the specification teaches that the methods may be used for generating transplantable organs and tissues and the generation of transgenic animals. The generation of protein production cell lines is also taught ((page 6, lines 9-11). Additionally, in describing delivery agents such as ultrasound, the specification teaches the use of ultrasound to deliver large nucleic acids *in vivo* for applications such as imaging, for example, in imaging the interior of a blood vessel such as described in US 5,676,151, referenced on page 23 (line 2). The instant application teaches methods that can be used to deliver large nucleic acids and furthermore, the specification teaches that these large nucleic acids introduced into cells can be detected by visible markers for purposes such as mapping biological events using marker genes (see for example page 8, lines 2-10). The specification also teaches the introduction of large nucleic acids into nuclear donor cells for use, for example, in the generation of transgenic animals (see for example page 6, lines 3-9,and also page 15, lines 1-3). Furthermore, the specification teaches methods for the screening and optimization of gene delivery including for *in vivo* and *ex vivo* applications and methods for using labelled nucleic acid molecules for such purposes (page 6, lines 13-17, page 7, lines 22-27, and also page 33, lines 3-30).

The specification teaches methods of nucleic acid delivery for use in, but not limited to, the above-mentioned fields, including types of delivery agents, methods of using these delivery agents to deliver a nucleic acid molecule to a cell, and methods of introducing nucleic acids to cell types and subjects, including *in vitro*, *ex vivo* and *in vivo* delivery methods. Each of these steps are described in detail in the specification. The specification describes the steps of the claimed methods for introducing a nucleic acid molecule into a cell by exposing the nucleic acid to a delivery agent, exposing the cell to a delivery agent, and contacting the cell with the nucleic acid molecule. Furthermore, the specification teaches the introduction of a nucleic acid molecule *in vitro*, *in vivo* and *ex vivo* as well as the introduction of a nucleic molecule into a cell of a subject that can be used for gene therapy as well as other applications.

The specification provides working examples of specific embodiments which encompass these steps such as: introduction of a nucleic acid molecule into cells, including cells in an animal subject; the use of energy and cationic compounds as delivery agents; the use of combinations of delivery agents; introduction of large nucleic acid molecules; and the introduction of nucleic acids into a variety of cell types. The specification also describes methods of labeling, detecting labeled nucleic acid molecules and measuring the delivery of nucleic acid molecules. Additionally, methods of gene expression and detecting gene expression from nucleic acid molecules delivered to cells are described.

A) Delivery Agents

The specification describes the introduction of nucleic acid into cells. For example, on page 17, line 22 through page 23, line 26, methods of DNA delivery and delivery agents are taught, such as the use of cationic lipids (page 20, line 3 through page 21, line 18) and cationic non-lipid compounds (page 21, line 19 through page 22, line 4), the use of ultrasound (page 22, line 9 through page 23, line 19) and electroporation (page 23, lines 20-26). Additionally, the specification teaches methods for contacting cells and nucleic acids with

delivery agents both separately and together (page 23, line 27 through page 28, line 9).

In addition, the working examples exemplify many delivery agents. Example 4 describes the introduction of Aces into Chinese Hamster lung fibroblast cells by contacting the DNA with the delivery agent Superfect and then contacting the Superfect:ACes complex with the cells (page 47, lines 7-13) and obtaining delivery of the Aces into the cells (page 47, lines 17-20 and page 47, line 28 through page 48, line 4). Examples 5 and 6 describe the introduction of GFP chromosomes using ultrasound and Saint-2 or ultrasound and Lipofectamine (pages 50-52). Tables 1 and 2 of Example 7 (pages 54-57) exemplify the use of numerous transfection protocols using delivery agents such as clonfectin, cytofectene, Eu-fectins, lipofectamine and superfect.

B) Methods for introducing DNA into specific cell types and subjects

The specification teaches that the delivery methods can be used in any cell type, tissue, organ or subject. For example, on page 8 (lines 28-30) the instant application teaches that the delivery methods can be used in both eukaryotic and prokaryotic cells, including primary cell lines, stem cells and embryonic cells. The working examples demonstrate delivery methods in a variety of cell types including Chinese Hamster lung fibroblast cells (Example 4, page 47, lines 1-20), LM (tk-)(Example 5, page 48, line 15 through page 49, line 31), CHO-KI, Hep G-2 and A9 cells (Example 6, page 50, line 1 through page 52, line 6).

1) *Ex vivo transfer*

Further, it is taught that methods for *ex vivo* therapy encompass transfecting cells *in vitro* followed by selection and then by introduction of cells into the body of a subject (page 16, lines 5-7). Methods for *in vitro* delivery are such as those discussed above. Additionally, the specification teaches selection of cells. For example, the specification teaches that nucleic acid molecules can produce a protein that confers drug-resistance, such as hygromycin (page 11,

lines 20-21). Drug-selection is further exemplified, *e.g.*, in Examples 4 and 7 for selecting transfected Chinese Hamster lung fibroblasts with hygromycin B (page 47, lines 16-18 and page 53, line 31 through page 54, line 4, respectively). Following selection, the specification teaches that cells can be introduced into a subject using such means as subcutaneous, intramuscular, intraperitoneal, intravascular and interlymphatic injection (page 25, lines 2-6). Additionally, cells can be administered using medical devices such as arthroscopes and catheters (page 25, lines 6-8). Such methods of administration are known to those of skill in the art.

2) In vivo transfer

The specification teaches methods for *in vivo* delivery of nucleic acid molecules. Examples of such methods taught by the specification include the application of delivery agents to the target cells in the body of a subject such as an organ, a tumor, a tissue or joint, followed by application of the nucleic acid molecules. For example, the specification teaches the application of a cationic compound to the target cells followed by the application of ultrasound and then the application of the nucleic acid for example, by injection (page 25, lines 10-21). It is also taught that the nucleic acid can be mixed with a delivery agent such as a cationic compound and delivered to the target area followed by ultrasound application to the target area (page 25, lines 22-25).

The specification teaches that targets for *in vivo* therapy include organs and tissues of the subject such as skin, muscle, stomach, intestine, lung, bladder, ovary, uterus, liver, kidney, pancreas, brain, heart, spleen, prostate and joints, for example, the knee, elbow, shoulder, wrist, hip, and finger (page 26, lines 1-5). Further, it is taught that primary cell lines can be used as targets for *in vivo* therapy, for example, fibroblast, muscle, stomach, intestine, lung, bladder, ovary, uterus, liver, kidney, pancreas, brain, heart, spleen, prostate (page 26, lines 5-8).

The specification also teaches methods of delivering nucleic acid molecules and cells to the target area of the body. For example, it is taught that with delivery agents such as energy, for example, ultrasound, that devices such as probes, needles, catheters, surgical tools and ultrasound baths as are known in the art can be used to administer the agent (page 22, line 19 through page 23, line 2). Additionally, the specification teaches for example that injection, instillation, cannulation, slow infusion and topical administration can be used as means of administration (page 26, lines 9-14). Further, methods of administration using medical devices are taught, for example using arthroscopes and catheters (page 26, lines 18-19). For example, a medical device such as an angioplasty balloon is coated with a liquid cationic compound formulation or a lyophilized mixture of a cationic lipid formulation and administered to the subject (page 26, lines 20-30).

C) Applications of the delivery methods for gene therapy

The specification describes the introduction of nucleic acid molecules by *ex vivo* and *in vivo* delivery for use in gene therapy. A definition of gene therapy is found on page 15, lines 18-22:

> As used herein, gene therapy involves the transfer or insertion of nucleic acid molecules, and, in particular, large nucleic acid molecules, into certain cells, which are also referred to as target cells, to produce specific gene products that are involved in correcting or modulating diseases or disorders.

Furthermore the steps of gene therapy are taught (page 15, lines 22-30):

> The nucleic acid is introduced into the selected target cells in a manner such that the nucleic acid is expressed and a product encoded thereby is produced. Alternatively, the nucleic acid may in some manner mediate expression of DNA that encodes a therapeutic product. This product may be a therapeutic compound, which is produced in therapeutically effective amounts or at a therapeutically useful time. It may also encode a product, such as a peptide or RNA, that in some manner mediates, directly or indirectly, expression of a therapeutic product.

The specification teaches methods for the application of *in vivo* and *ex vivo* delivery to gene therapy. For example, the specification describes factors that will influence dosage such as mode of administration, age and weight of the subject and the delivery agents used. Further, the specification teaches weight ratios of cationic compounds and nucleic acids and provides guidance for their administration to subjects (page 27, line 18 through page 28, line 4). The specification additionally teaches that the methods can be used either systemically or locally in a subject. For example, the methods can be applied locally to an affected joint for treatment of rheumatoid arthritis, psoriasis or diabetes (page 28, lines 5-6) and that delivery agents such as ultrasound can be applied to a joint using contrast reagents to focus lithotripter shock waves at a defined site (page 23, lines 15-19).

The Office Action alleges that the specification fails to provide any other practical utility for *ex vivo* or *in vivo* gene transfer other than gene therapy. As discussed above, however, gene therapy is only **one** application of the methods as claimed and as taught in the specification. As discussed above, the specification teaches numerous applications besides gene therapy for use with the claimed methods such as production of proteins, generation of transgenic animals, uses with imaging methods for medical devices, mapping biological events *in vivo*, gene delivery optimization technology development, generation of transplantable organs and tissues, in addition to *in vivo* and *ex vivo* therapies.

Thus, in light of the numerous applications in medical, pharmaceutical, biological and technology-related research fields for the instantly claimed methods of introducing a nucleic acid molecule into a cell *in vitro*, *in vivo* and *ex vivo*, the importation of "gene therapy" as a limitation of the claims is unfounded. The claims are to methods of nucleic acid delivery, not methods of gene therapy. Applicants respectfully point out that the importation of limitations from the specification into the claims is improper. As has been stated in the recent case Amgen v. Hoechst Marion Rousel, 314 F.3d. 1313

(Fed. Cir. 2003), it is improper "to add extraneous limitations to a claim, that is limitations added wholly apart from any need to interpret what the patentee meant by particular words or phrases in the claim. The danger of improperly importing a limitation is even greater when the purported limitation is based upon a term not appearing in the claim." Furthermore, the court quoted "If we once begin to include elements not mentioned in the claim in order to limit such claim..., we should never know where to stop " from Johnson Worldwide Assoc., Inc v. Zebco Corp., 175 F.3d 985, 990, 50 USPQ2d 1607, 1610 (Fed. Cir. 1999)(quoting McCarty v. Lehigh Val. R.R., 160 U.S. 110, 116 (1895)).

Although Applicant objects to the emphasis the Examiner has placed on gene therapy in the interpretation of the claims, as discussed above, Applicant respectfully submits that gene therapy as an application of the claimed nucleic acud delivery methods is taught.

Thus, the specification teaches one skilled in the art how to introduce a nucleic acid molecule into a cell by exposing the cell to a delivery agent, exposing the nucleic acid molecule to the delivery agent, and contacting the cell with the nucleic acid molecule, and the various further embodiments of the methods including applications in gene therapy as described and claimed.

Knowledge of those of skill in the art

At the time of filing of the instant application and before, a broad body of knowledge had amassed in the delivery of nucleic acids to cells and the application of nucleic acid delivery and gene transfer to such methods as gene therapy, imaging, organ transplantation, protein production and the generation of transgenic animals.

Agents for delivering nucleic acid molecules and sources therefor are described in the specification. For example, reference is made to electroporation methods and apparatuses *e.g.*, in U.S. Patent Nos. 6,027,488, 5,993,434, 5,944,710, 5,507,724, 5,501,662, 5,389,069, 5,318,515 (page 23, lines 20-26). Procedures and methods for ultrasound systems also are provided. These

include systems described in International PCT application No. WO 99/21584 and U.S. Patent No. 5,676,151 (page 22, line 10 through page 23, line 2). The instant application references chemical delivery agents that can be used in the methods provided. These include well-known agents, such as cationic reagents such as Lipofectin, LipofectAMINE, and LipofectAMINE PLUS from Life Technologies, Inc., (Burlington, Ont., *see* U.S. Patent No. 5,334,761 and 5,736,392; *see, also* U.S. Patent No. 6,051,429), Effectene (Qiagen, Inc., Mississauga, Ontario) (Non liposomal lipid formulation), Metafectene (Biontex, Munich, Germany) (Polycationic lipid), Eu-fectins (Promega Biosciences, Inc., San Luis Obispo, CA) and numerous other cationic lipids (page 20, line 24 through page 21, line 18), as well as non-lipid compounds such as SUPERFECT™ (Activated dendrimer (cationic polymer:charged amino groups); Qiagen, Inc., Mississauga, ON) and CLONfectin™ (Cationic amphiphile N-t-butyl-N'-tetradecyl-3-tetradecyl-aminopropionamidine; Clontech, Palo Alto, CA) (page 21, line 20 through page 22, line 4). Procedures for calcium-phosphate-mediated delivery are referenced such as Graham *et al.* (1978) *Virology 52*:456-457; Wigler *et al.* (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*:1373-1376; and (1990) *Current Protocols in Molecular Biology*, Vol. 1, Wiley Inter-Science, Supplement 14, Unit 9.1.1-9.1.9 (page 41, lines 1-5).

The specification further provides references for preparing large nucleic acids such as artificial chromosomes for use in the claimed methods. For example, at page 11, lines 14-15, reference is made to the disclosures of U.S. Patent Nos. 6,025,155 and 6,077,697 and International PCT application No. WO 97/40183.

Procedures relating to the use of reporter genes for detecting gene products encoded by the delivered nucleic acid also are referenced and described in the instant application. For example, references are cited for reporters such as fluorescent protein, CAT (chloramphenicol acetyl transferase) (Alton and Vapnek (1979), *Nature 282*: 864-869) luciferase, *e.g.*, firefly

luciferase (deWet *et al.* (1987), *Mol. Cell. Biol. 7*: 725-737); bacterial luciferase (Engebrecht and Silverman (1984), *PNAS 1*: 4154-4158; Baldwin *et al.* (1984), *Biochemistry 23*: 3663-3667); and alkaline phosphatase (Toh *et al.* (1989) *Eur. J. Biochem. 182*: 231-238, Hall *et al.* (1983), *J. Mol. Appl. Gen. 2*: 101) (page 16, lines 12-23).

Additionally, agents for detection of labelled nucleic acids delivered by the claimed methods are referenced in the instant application including, for example, Pittman *et al.*, *J Immunol Methods 103*: 87-92 (1987) (page 28, lines 16-20) and Gratzer *et al.* Cytometry (1981) 6:385-393 (page 55, lines 8-9).

These references to numerous published references and sources for agents for delivery of nucleic acids into cells, preparation of large nucleic acids for use with the instantly claimed methods, methods for the detection and analysis thereof for expression of encoded genes, including reporter gene products, demonstrate the large volume of information available at the time of filing of the instant application and thus evidence the advanced state of the art at the relevant time. The instant application details their application to the claimed and disclosed methods.

Presence of Working Examples

The specification provides numerous working examples and descriptions of methods of delivering nucleic acids such as: introduction of a nucleic acid molecule using specific ordered steps; the use of energy and cationic compounds as delivery agents; the use of combinations of delivery agents; introduction of large nucleic acid molecules; and the introduction of nucleic acids into a variety of cell types. The specification also describes methods of labeling, detecting labeled nucleic acid molecules and measuring the delivery of nucleic acid molecules. Additionally, methods of gene expression and detecting gene expression from nucleic acid molecules delivered to cells are described. As detailed above, an example of the preparation of large nucleic acids for use with the delivery methods of the instant application is given in Example 1 (pages

35-45). Delivery of nucleic acids is exemplified in Examples 4-7. Example 4 describes the introduction of ACes into Chinese Hamster lung fibroblast cells using the delivery agent Superfect and then contacting the Superfect:ACes complex with the cells (page 47, lines 1-20 and page 47, line 28 through page 48, line 4). Examples 5 and 6 describe the introduction of GFP chromosomes using ultrasound and Saint-2 or ultrasound and Lipofectamine (pages 50-52) into a variety of cell types such as CHO-KI, Hep-G2, A9 and V79-4 cells. Tables 1 and 2 of Example 7 (pages 54-57) exemplify the use of numerous transfection protocols using delivery agents such as clonfectin, cytofectene, Eu-fectins, lipofectamine and superfect. Measurement of gene expression from delivered nucleic acids is exemplified in Example 1 (page 41, lines 1-25), Example 5 (page 48, line 15 through page 49, line 31). Example 6 (page 50, line 1 through page 51, line 4) and Example 7 (page 54, lines 4-6). Detection of the delivery of nucleic acids by detecting labelled cells is exemplified in Example 4 (page 47, line 22 through page 48, line 4) and Example 7 (page 55, line 8 through page 56, line 5 and also Table 2, pages 56-57).

Predictability

As is known to those of skill in the art (discussed above), the level of knowledge and skill in the delivery of nucleic acids into cells as claimed in the instant application was high as of the effective filing date. Therefore, given the extensive teachings of the specification, in combination with what was known at the time the instant application was filed, it is not unpredictable that nucleic acid molecules, including large nucleic acid molecules can be delivered to cells according to the steps of the instant methods. Further, the use of such delivery methods in *e.g.*, gene transfer for gene therapy, is not unpredictable.

The pending claims are directed to methods of delivering a nucleic acid molecule into a cell by exposing the nucleic acid and/or the cell to a delivery agent(s). Having described the claimed methods, and detailed the numerous

agents and procedures for effecting each step in the methods in the application, there is no issue of predictability in the instant case.

The Office Action alleges that the state of the art in regards to *ex vivo* or *in vivo* gene therapy, at the time of filing of the instant application, was considered to be highly unpredictable, regardless of the mode of delivery. The Office Action cites the following references in support the allegation of unpredictability with respect to gene therapy: Verma *et al.* (1997) Nature 389:239-242; Marshall (1995) Science 269:1050-1055.; Orkin *et al.*, (1995) Report and Recommendations of the panel to assess NIH investment in research on gene therapy; Eck *et al.*, (1996) Goodman and Gilman's *The Pharmacological Basis of Therapeutics*, Chapter 5; Ross *et al.*, (1996) *Human Gene Ther.* 7: 1781-1790; Rubanyi (2001) *Mol. Aspects Med.* 22:113-142; Schwaab *et al.*, (2001) *Semin. Thromb. Hemost.* 27:417-424; Rissanen *et al.*, (2001) *Eur. J. Clin. Invest.* 31: 651-666; and Emanueli *et al.*, (2001) *Br. J. Pharmacol.* 133:951-958.

First, Applicant respectfully submits that the question of whether the instant claims satisfy the requirements of 35 U.S.C. § 112, first paragraph, does not turn on the predictability/unpredictability of the art of gene therapy. The pending claims are directed to methods of nucleic acid delivery to cells, and the steps of these methods are set forth and taught in the specification in great detail. The claims are not directed to general methods of curing disease or to general methods of gene therapy *per se*. As discussed above, the steps of the claimed methods are elucidated in great detail in the form of descriptions throughout the specification and in working examples so that they may be optimized for particular applications such as gene therapy.

A significant portion of the grounds for the rejection of the claims under 35 U.S.C. § 112, first paragraph, is based on the alleged unpredictability of the art of gene therapy in general. As explained above, however, the issue of whether the specific instant claims are enabled by the specification should not

turn on the state of the art of gene therapy as generally discussed at pages 6-11 of the Office Action. Instead, the relevant question with regard to enablement of the subject matter of the instant claims is whether the particular steps and materials of the claimed methods are described in the specification in such a way as to enable one skilled in the art to make and use the subject matter **as claimed.**

Further, Applicant notes that several of the cited references are post-filing date publications, Rubanyi *et al.* (June 2001), Rissanen *et al.* (August 2001), Emmanueli *et al.* (August 2001) and Scwaab *et al.* (August 2001). Applicant respectfully submits that reliance upon post-filing date references to establish a lack of enablement is improper.

Furthermore, notwithstanding the above and contrary to the Examiner's assertions, Applicant respectfully submits that none of the cited references have any bearing on the predictability of the methods as instantly claimed, including their application to gene therapy. Each of the cited references is discussed in turn below:

Verma *et al.* The Office Action alleges that Verma *et al.* (1997) *Nature 389*:239 teaches that gene delivery is the "Achilles heel" of gene therapy and that most of the approaches suffer from poor gene delivery and transient gene expression.

Verma *et al.* reviews the state of the art of gene therapy as of 1997 and identifies prospects for its future by identifying future areas of research emphasis. Applicant respectfully asserts that the state of the art of gene therapy in 1997 has no bearing on the predictability of delivering a nucleic acid molecule to a cell for gene therapy or any other purpose as of the filing date, March 2001, of the instant application. Furthermore, one of the key problems to be solved as of 1997 as pointed about by Verma *et al.* is gene delivery, the very methods the instant application addresses. Thus, the instant application teaches how to overcome the limitations described in Verma *et al.* (see instant

specification, *e.g.*, at Table 2 of Example 7, pages 56-57, demonstrating efficient delivery of nucleic acid molecules into cells).

With regard to gene expression, Verma *et al.* specifically addresses the problems associated with using retroviral vectors in gene delivery, not gene expression obtained by gene transfer in general (*see* page 240, paragraph spanning col 1-2). The instant application teaches nucleic acid delivery and gene transfer to cells using methods that are successful in achieving gene expression in the cells (see, for example, Example 5, page 59, lines 26-31 and Example 6, page 51, lines 22-23). Thus, the instant application also overcomes the limitations of Verma *et al.* pertaining to gene expression.

Marshall *et al.* The Office Action alleges that Marshall *et al.* (1995) *Science 269*:1050-1055 teaches that there are difficulties associated with transferring genes to target cells and that "many problems must be solved before gene therapy will be useful for more than rare applications" (page 1055, col.1, ¶1).

The Marshall *et al.* publication is a review of the early years of gene therapy that, like Verma *et al.*, identifies areas for future investment and research. Applicant again respectfully submits that the state of the art of gene therapy in 1995 has no bearing on the predictability of the instantly claimed methods of nucleic acid delivery. Most gene therapy trials were just beginning in 1995 and most trials were still in Phase I, which tests safety and not efficacy (page 1054, col. 2, ¶2).

Further, Marshall *et al.* specifically addresses problems associated with agents such as various viruses and the carrier PEG (polyethylene glycol) in obtaining efficient gene delivery and expression. The teachings of Marshall *et al.* have no bearing on the success of nucleic acid delivery and gene transfer methods as instantly claimed. In fact, Marshall *et al.* describes the use of cationic lipids as the most promising alternative to the viral vector delivery agents that were widely in use at the time. Marshall *et al.* quotes one "skilled

researcher" in the field of cationic liposome-mediated delivery, Gary Nabel of the Howard Hughes Medical Institute, as predicting the dramatic improvement in such technology over the "next few years", increasing levels of gene expression by at least an order or magnitude. Therefore, Marshall *et al.* in fact predicts the future success of methods as instantly claimed, rather than support their unpredictability.

To evidence the progress and success of the field of gene therapy between the publication of Marshall *et al.* and the filing date of the instant application, Applicant provides Mountain (2000) *TIBTECH 18*: 119-128 (a copy is provided in a Supplemental Information Disclosure statement being filed on the same day herewith). Mountain reviews the first decade of gene therapy, providing a comprehensive view of the field at a time that is closer to the filing date of the instant application than many of the review articles cited in the Office Action. Mountain states "Within the past year, clinical benefit from gene therapy has been clearly demonstrated for the first time" (page 120, col. 2 paragraph bridging to page 121). The author further states, "the most important recent development in gene therapy is the clear demonstration of efficacy in clinical studies, which might lead to a restoration of public and investor confidence in gene therapy" (page 126, col. 2, last paragraph). Thus, gene therapy had clearly achieved success beyond the boundaries described by Marshall *et al.* in 1995. Furthermore, as one of the limitations in gene therapy, Marshall *et al.* cites gene transfer as a key problem in the field (page 1054, col. 3, ¶2). The instant application addresses this limitation and teaches methods to overcome that limitation, providing methods for efficient transfer of nucleic acid molecules to cells as taught in the specification and exemplified in the working examples (see, for example, Table 2 of Example 7, pages 56-57).

Orkin *et al.* The Office Action cites Orkin *et al.* (1995) Report and Recommendations of the panel to assess NIH investment in research on gene therapy, which allegedly teaches that the available vector systems are

unsatisfactory and have not been experimentally validated and furthermore that clinical efficacy "has not been definitively demonstrated at this time in any gene therapy protocol" (page 1, ¶3).

Applicant again respectfully points out that the state of the art of gene therapy in 1995 has no bearing on the predictability of the instantly claimed methods. Moreover, Orkin *et al.* acknowledges that the future of gene therapy is "great" (*see* page 1, para 2.) and merely identifies parameters that govern efficient implementation of gene therapy methods. The fact that with respect to methods of gene therapy, the NIH identifies well-studied and characterized limitations of the art, as determined through years of research, that need to be funded in order to promote continued advancement in gene therapeutic strategies, make the methods all the more predictable in terms of the future direction to follow.

It is respectfully submitted that the Examiner has engaged in a selective reading of Orkin *et al.*, resulting in a mischaracterization of the reference that cannot validly be relied on to support an allegation of unpredictability of gene therapy. Orkin *et al.* strongly recommends funding of gene therapy research because of its "great promise", and urges the NIH to maintain support for peer reviewed research in gene therapy (*see* page 18, "Recommendations" para 2.). Orkin *et al.* further acknowledges the ample resources in terms of skilled personnel and clinical research facilities that were available as of 1995 to ensure progress in the field (page 18, last sentence to page 19, "Recommendations", para 1.).

Although Orkin *et al.* identifies particular problems, such as lack of collaborative research, lack of consistent adherence to high standards in gene therapy clinical protocols and insufficient focus on basic aspects of gene transfer, it clearly had been demonstrated, based on actual clinical trial data, that therapeutically relevant genes could be transferred into human patients and

be expressed within the patient in such a manner as to show biologic efficacy (page 12, fourth full paragraph beginning with "Only a few...").

Applicant is not aware of any requirement under current U.S. patent law specifying particular minimum levels of optimization and certified efficacy in order for a treatment-related area of art to qualify as sufficiently "predictable" such that lack of enablement under 35 U.S.C. § 112, first paragraph, is not a consideration. The relevant standard is not that of an established, fully optimized, clinical course of treatment; rather, even in an *unpredictable* art, a patent application satisfies the requirements of 35 U.S.C. § 112, first paragraph, as long as it provides sufficient disclosure, either through illustrative examples or terminology, to teach those of ordinary skill how to make and use the claimed subject matter with reasonable, but not undue, experimentation. There is no requirement that a treatment method achieve a specified level of efficacy or efficiency in order to be considered "enabled" by the specification.

As discussed above, Orkin *et al.* strongly advocates progress in gene therapy and provides specific recommendations for future NIH-sponsored gene therapy research. Some of these recommendations have in fact been addressed and successfully accomplished in the instant application.

For example, Orkin *et al.* points out that gene therapy as of 1995 was hindered by a low frequency of gene transfer (see for example page 2, paragraphs 2 and 4, page 8, paragraph 4, page 13, paragraph 3). Orkin *et al.* further points out that many of these problems are associated with the use of retroviral vectors for gene delivery, and recommends direct administration of DNA or DNA-liposome complexes as being "attractive" approaches for future gene therapy research (page 8, third full paragraph). Thus, the limitations and proposed future directions of gene transfer described in Orkin *et al.* have been addressed in the instant application, which provides nucleic acid delivery methods that include cationic lipid delivery agents in some embodiments (for

example, such teachings are exemplified in Example 4, pages 46-48, Examples 5 and 6, pages 48-52 and Example 7, pages 52-57).

Orkin *et al.* further states that special emphasis should be placed on vector development such as artificial chromosomes (page 11, ¶4). The instant application provides artificial chromosomes that can be used with the claimed methods of gene delivery (page 11, pages 8-24) and provides methods to introduce these nucleic acid molecules into cells (*see*, for example, the delivery methods taught in pages 17-23 and Examples 4-7, pages 46-57 which each exemplify delivery methods using artificial chromosomes such as ACes).

The second statement cited from Orkin *et al.* by the Examiner, that clinical efficacy "has not been definitively demonstrated at this time in any gene therapy protocol" (page 1, ¶3), also does not support an allegation that gene therapy was unpredictable as of the time of filing of the instant application. The majority of the trials covered in the NIH report are from 1993-1994 (see pages 19-20), again not the state of the art as of the filing of the instant application in March 2001. Further, as discussed above, Orkin *et al.* acknowledged the existence of several "elegant" clinical studies and merely pointed out that more such well-designed clinical studies were necessary to further advance the state of the art of gene therapy (page 12, fourth full paragraph beginning with "Only a few..."). Thus, the steps to be followed in order to conduct well-designed clinical studies were known and acknowledged (*i.e.*, predictable). The feasibility of the clinical studies was not questioned, it was only a need for more <u>numbers</u> of such studies in order to obtain more data that was identified in Orkin *et al.*

To further rebut the assertion that clinical efficacy had not been achieved as of the filing date of the instant application, Applicant respectfully submits Cavazzana-Calvo *et al.* (2000) *Science 238*:669-672 (a copy is provided along with a Supplemental Information Disclosure statement being filed on the same day herewith). The publication of Cavazzana-Calvo *et al.* describes the clinical efficacy achievements in gene therapy. This reference describes the use of

gene therapy to successfully treat human severe combined immunodeficiency (SCID)- X1 disease. *Ex vivo* gene therapy demonstrated clinical efficacy in the two patients treated including long-term gene expression. Thus, Cavazzana-Calvo *et al.* demonstrates that at the time of filing, gene therapy had been successfully demonstrated in clinical trials and had reached a level of predictability.

Eck *et al.* The Office Action cites Eck *et al.* (1996) Chapter 5 of Pharmacological Basis of Therapeutics (pages 77-101) as teaching that there are numerous factors to overcome in gene therapy such as the fate of the DNA vector, the *in vivo* consequences of gene expression, the fraction of vector taken up by the cell, and the stability and level of the RNA and protein produced.

It is respectfully submitted that such a selective reading of Eck and Wilson, in which statements regarding the state of gene therapy in 1996 are taken out of context, has resulted in a mischaracterization of the reference that cannot validly be relied on to support an allegation of unpredictability of gene therapy. For example, the abstract of Eck and Wilson mentions how gene therapy has overcome barriers of the former cellular inaccessibility of large proteins encoded by therapeutic genes. The article provides several examples of the progress made in developing delivery systems for gene therapy, its applications not only in inherited single-gene defects, but also in acquired illnesses such as cancer, cardiovascular and infectious diseases, and the continual advancement for strategies to introduce recombinant DNA into tissues in a selective manner.

Although Eck and Wilson acknowledge that gene transfer was not an established clinical treatment regime, it clearly had been demonstrated, *based on actual clinical trial data*, that therapeutically relevant genes could be transferred into human patients and be expressed within the patient in such a manner as to show biologic efficacy. Eck and Wilson further provides a summary of the

studies demonstrating that transfer of genes to humans is feasible (see Table 5-1, pp. 80-81) and statistics concerning the numbers and outcomes of human gene transfer studies. Eck *et al.* cites numerous positive examples of gene therapy success as demonstrations that these obstacles are overcome. For example: 1) *Ex vivo* treatment with the LDL receptor demonstrated feasibility, safety and potential efficacy of ex vivo treatment (page 92, col. 2, ¶2); 2) Adenovirus delivery of CFTR normalize chloride conductance (page 93, col. 2, ¶2); 3) Ectopic expression of FGF-1 and TGFβ-1 produced vascular changes (page 94, col. 1, ¶4); 4) retroviral delivery produced a 200-1000X increase in sensitivity to ganciclovir (page 95, col. 1, ¶4); and 5) activation of the host immune system by ectopic expression of B7 to recognize and eradicate tumor cells (page 96, col. 2 ¶1 and 2). Further, Eck *et al.* teaches many successful examples of non-viral delivery (see especially pages 90-91) including mouse muscle expression up to 60 days with no adverse inflammatory response (addressing the obstacles of gene expression, stability and safety), gene gun delivery to skin and skin tumor ideal for gene mediated immunization, demonstration of intravenous and airway delivery of cationic liposomes, tumor attenuation after delivery of a histocompatibility gene by plasmid liposome delivery, and rabbit prostanoid synthesis restored following gene delivery with plasmid liposomes.

Like Orkin *et al.*, Eck *et al.* establishes that methods for gene therapy are known in the art but that there are areas for improvement. Eck *et al.* summarizes on page 99, "the clinical application of gene therapy is more limited by the availability of suitable gene transfer methodology ..."(col. 2, ¶2). As discussed above, the instant application remedies this limitation by providing methods for efficient gene delivery of nucleic acid molecules.

Eck and Wilson concludes that human gene therapy, although still in its infancy, offers the possibility for "major advancements in the prevention and

treatment of many diseases". They also conclude that as "increasing numbers of investigators address these issues, better reagents likely will emerge".

As discussed above, Applicant is not aware of any requirement under current U.S. patent law specifying particular minimum levels of optimization and certified efficacy in order for a treatment-related area of art to qualify as sufficiently "predictable" such that lack of enablement under 35 U.S.C. § 112, first paragraph, is not a consideration. The relevant standard is not that of an established, fully optimized, clinical course of treatment; rather, even in an *unpredictable* art, a patent application satisfies the requirements of 35 U.S.C. § 112, first paragraph, as long as it provides sufficient disclosure, either through illustrative examples or terminology, to teach those of ordinary skill how to make and use the claimed subject matter with reasonable, but not undue, experimentation. There is no requirement that a treatment method achieve a specified level of efficacy or efficiency in order to be considered "enabled" by the specification.

Contrary to the position set forth in the Office Action, Eck and Wilson's assessment of the state of the art of gene therapy is that therapeutic gene transfer to humans has been proven to be feasible, as borne out by successes in gene transfer clinical trials, and its accomplishments to date at the time of publication were impressive.

It appears that the Examiner, in asserting the unpredictability of the art of gene therapy, has equated "limitations" with "unpredictability." It is respectfully submitted that although methods of gene therapy may be associated with certain limitations and limited success, this does not establish the art as unpredictable. In fact, with respect to methods of gene therapy, the well-studied, -identified and -characterized limitations of the art, as determined through years of research and, as Eck and Wilson *et al.* report, several clinical trials, make the methods all the more predictable. The practitioner is well aware of the potential obstacles and clearly knows what he or she is up against in

designing and carrying out such therapeutic methods. As such, it is respectfully submitted, that although the art of gene therapy may not have been a routine, clinical practice at the effective filing date of the subject application, it was not so unpredictable as to qualify as a major factor in the determination of whether the requirements of 35 U.S.C. § 112, first paragraph, are satisfied with respect to the instantly claimed subject matter.

Verma *et al.* and Ross *et al.* The Office Action further alleges sustained gene expression is unpredictable in gene therapy, citing Verma *et al.* (*supra*) as teaching that the search for enhancer-promoter combinations is trial and error and citing Ross *et al.* (1996) *Human Gene Ther. 7*:1781-1790 as teaching that no correlation exists between successful expression of a gene and a therapeutic result.

Applicants respectfully point out that these statements have both been presented out of context from their respective references and furthermore, that neither reference (published 1996 and 1997, respectively) represents the state of the art of gene therapy as of the filing of the instant application. The statement of Verma *et al.* is directed at the specific use of retroviral vectors, not gene therapy in general (page 240, col. 2, ¶2). As of the filing date of the instant application (March 2001), numerous enhancer-promoter combinations were known and the state of the art with respect to such gene expression tools have advanced since the publication of Verma *et al.* in 1996. The instant application provides methods to easily and quickly test many different nucleic acid molecules for gene expression, thus remedying this limitation of Verma *et al.* Further, the instant application demonstrates that the introduced nucleic acid molecules provide gene expression in a variety of different cell types again overcoming the limitations cited in Verma *et al.* (*see* for example, pages 50-52 of the instant specification, which exemplifies GFP expression in ovary, liver and fibroblast cells).

The publication of Ross *et al.* is a review of the early years of gene therapy, primarily of viral and retroviral-mediated gene therapy. Ross *et al.* clearly teaches that as of its publication date, most gene therapy trials were in Phase I with the goal of establishing safety rather than efficacy (page 1789, col. 1, 1st full paragraph). Like Orkin *et al.* and Eck *et al.*, Ross *et al.*, states "there remains a need for a further effort at improving the basic technology" (page 1789, col. 1, 1st full paragraph). This statement is followed by a summary of the findings of Orkin *et al.* from the NIH report (*supra*) and thus in a similar manner, Ross *et al.* lists specific areas for future improvement and investment that can be predictably implemented. Thus, Applicant respectfully submits that gene therapy is not unpredictable according to Ross *et al.* but rather a constantly improving field of research. This is further evidenced by the publications of Mountain and Cavazzana-Calvo *et al.*, discussed above, which demonstrate the improvements in gene therapy subsequent to Ross *et al.* and as of the filing date of the instant application.

Rubanyi *et al.* The Office Action further alleges that Rubanyi (2001) *Mol. Aspects Med 22*:113-142 teaches that problems with gene therapy, such as gene delivery and improvement in gene expression control systems, had yet to be overcome at the time of filing of the instant application.

Rubanyi *et al.*, like the references discussed above, reviews the field of gene therapy by identifying the obstacles that have been faced by researchers and progress made and successes achieved as of 2001. Rubanyi *et al.* states that previous problems in gene therapy methods stemmed from existing technologies (for understanding the molecular basis of human disease and for construction of suitable gene delivery vectors) not being able to meet the practical needs of clinical trials for gene therapy. Rubanyi *et al.* then goes on to state that "over the past years, significant progress was made in various enabling technologies", including gene delivery methods (see Abstract; emphasis added). Rubanyi *et al.* further points to many promising areas for

gene therapy, including hemophilias and cardiovascular diseases. Rubanyi *et al.* acknowledges that gene therapy was at a developmental stage, and provides specific technical hurdles that needed to be addressed to further advance the field, but does not doubt such advancement. For example, at page 136, Rubanyi *et al.* states, "As with all new technologies, gene therapy has to run its course in its present 'development' phase before it reaches 'maturation', when its full potential will be exploited." It is respectfully submitted that failure to reach the full potential of a particular technology, gene therapy or otherwise, does not characterize the technology as "unpredictable."

Rubanyi *et al.* does not question the feasibility of gene therapy, the predictability of the basic methods employed therein, nor the nature of the protocols and specific steps to be developed toward its advancement. Rubanyi *et al.* only identifies limitations in gene therapy, and ways in which the limitations may be overcome. Thus, like the references above, Rubanyi *et al.* is a review of how science in any area, including gene therapy, while feasible and involving predictable technologies, must continually be improved upon. In fact, as discussed above, Rubanyi *et al.* acknowledges the advancement in methods of gene delivery, thus making the instantly claimed methods of nucleic acid delivery all the more predictable.

Further, contrary to the allegation that Rubanyi *et al.* teaches the unpredictability of gene therapy, the authors cite a number of successes of gene therapy as of 2001, for example with cardiovascular disease (page 129, section 4.2), gene transfer of VEGFs and FGFs have resulted in improved blood flow and collateral development in animal models. Successes are also cited for gene transfer of immune stimulatory genes such as IL-2, IL-12 and IFNs in animal models and in initial clinical studies (page 128, ¶2). Thus, the need for improvements, which is a constant need in any field, does not signal lack of enablement but rather the search for betterment of an already established art.

The Examiner cites an additional statement of Rubanyi *et al.* from page 131 (third full paragraph) that teaches hurdles must be overcome before gene therapy is successful in the clinic. Applicant respectfully submits that this statement is taken out of context. The statement, which is in a section entitled "Success factors and their fulfillment in therapeutic angiogenesis using gene therapy" is set forth in the context of how one may overcome technical hurdles in clinical trials by selecting a disease target of interest such as CAD and then examining all of the success criteria such as availability of therapeutic genes, gene delivery, gene expression, lack of unwanted reactions to therapy, clinically relevant effect in animal models, appropriate safety in Phase I and significant therapeutic effect in Phase II and Phase III clinical trials. The authors conclude that each of these criteria have already been met and fulfilled "with the exception of demonstrating statistically significant clinical efficacy in a large scale, multicenter, double-blind and placebo controlled trial..."(section 4.2.2; paragraph spanning pages 131-132). Thus, Rubanyi *et al.* teaches the success of the basic methods of gene therapy, the limitations that must be overcome, and the available tools to overcome the limitations. Contrary to the Examiner's assertion, the teachings of Rubanyi *et al.* demonstrate the feasibility of gene therapy methods and further demonstrate that it is possible to predictably and systematically address limitations of gene therapy to improve its efficacy.

Schwaab *et al.* The Office Action also alleges that Schwaab *et al.* (2001) *Seminars in Thrombosis and Hemostasis 27(4)*:417-424 teaches that gene therapy studies with factor VIII and factor IX were complicated and that gene therapy is not established for hemophilia.

Schwaab *et al.* is a focussed review of gene therapy for treating haemophilia patients. Applicants respectfully assert that the methods as claimed are methods of gene delivery for use in many areas, including gene therapy. The instant methods are not methods of gene therapy *per se*, much less gene therapy involving particular genes for a particular disease, hemophilia.

The particular complications of a single study with specific genes for factors VIII and IX have no bearing on the predictability of the instantly claimed methods.

Further, Schwaab *et al.* explains that the difficulties in the hemophilia studies are due to a specific gene defect associated with this type of hemophilia, a large gene deletion, which activates the immune system in response to exogenously administered factor IX, not due to a general problem with gene therapy or gene delivery methods (page 420, col. 1 ¶3). Schwaab *et al.* additionally cites many positive results in the field of gene therapy for hemophilia such as, for example, successful *ex vivo* therapy in rabbits and dogs (page 419, col. 2 ¶2), long-term gene expression in mice models (page 420, col. 1 ¶5 and col. 2, ¶2), and therapeutic effects on blood coagulation in dogs (page 420, col. 2, ¶4 through page 421, col. 1 ¶1).

Rissanen *et al.* The Office Action further cites Rissanen *et al.* (2001) *Eur. J. Clinical Invest. 31:*651-666 as teaching that although gene therapy of ischemic disorders have established proof of principle, many important questions remain to be addressed.

Applicants respectfully assert that this summary statement of the Examiner points to the success of gene therapy and not to evidence of its "unpredictability." Rissanen *et al.* is a review of gene therapy treatments for vascularization of lower limbs. Rissanen *et al.* presents data showing successful gene therapy using Ang-1 and VEGF factors (page 654, col. 2), a variety of delivery systems including adenovirus, HSV, AAV, plasmid DNA with and without carrier and baculovirus (page 655, paragraph spanning col. 1-2) and success in clinical studies (page 657, col 2. final paragraph).

The statement cited by the Examiner appears in the final section entitled "Conclusions and future directions" and is meant to point to future areas of study, now that proof of principle has been established. One improvement needed according to Rissanen *et al.* is gene transfer efficiency, the very improvement addressed and accomplished by the instant application. Thus

Rissanen *et al.* demonstrates that gene therapy is established in the art and the instant application provides methods that contribute to the further advancement in this field. As discussed above, Applicant is aware of no requirement that for a technology to be predictable, it must not require any further progress. The relevant standard is not that of an established, fully optimized technology but a sufficient disclosure, either through illustrative examples or terminology, to teach those of ordinary skill how to make and use the claimed subject matter with reasonable, but not undue, experimentation.

Emanueli *et al.* The Office Action cites Emanueli *et al.* (2001) *Brit. J. Pharmacology 133*:951-958 as teaching that delivery of angiogenic inducers has been successful but that obstacles still remain as of the effective filing date of the instant application.

Like Rissanen *et al.* and Schwaab *et al.*, this publication is a focussed review of gene therapy in a specific field, namely, angiogenesis. Applicants respectfully submit that contrary to the Examiner's assertion that this reference demonstrates the "unpredictability" of gene therapy, Emanueli *et al.* in fact teaches the success of gene therapy in vascular therapy. For example, on page 952 (col. 2, ¶3) Emanueli *et al.* cites a study by Leiden *et al.* demonstrating successful protein expression from delivery of angiogenic GF. At page 953, the authors cite studies by Kay *et al.* and Isner *et al.* demonstrating stable expression of marker genes in haemopoietic progenitor cells and successful *ex vivo* therapy in bone marrow cells, respectively (col. 1, ¶4). At page 954, Emanueli *et al.* teaches that a single intra-muscular injection of human tissue kallilrein (HK) was effective in preventing microvascular rarefaction and that Isner *et al.* have demonstrated that VEGF165 therapy improves limb perfusion (col. 1 ¶2-4). Emanueli *et al.* also cites Losordo *et al.* (page 954 col 1 ¶5), Safi *et al.* (page 956, col. 1, ¶2), Kalka *et al.* and Shintani *et al.* (page 956, col 2 ¶2) for successful demonstration of therapies in model animals.

The statement regarding abnormal vasculature formation that the Examiner refers to on page 955 of Emmanueli *et al.* is in reference to specific studies with VEGF and appears under the heading of "Future directions to improve effectiveness and safety of vascular gene therapy". This is similar to the statement in Rissanen *et al.* as well as other references discussed above, such as Orkin *et al.* and Eck *et al.* Each of the references describe or establish proof of principle for gene therapy and simply strive to point out future directions for further improvements. Again, Applicant respectfully submits that the standard for "predictability" is not that of a completely optimized method or technology, requiring no advancement.

Summary of References and their applicability to predictability of gene therapy

As discussed above, the articles cited in the Office Action demonstrate successes within the field of gene therapy, and provide specific parameters that must be addressed for its advancement. The success of gene therapy is further supported by the publications of Mountain and Cavazzana-Calvo *et al.*, cited herein, which clearly demonstrate that clinical efficacy of gene therapy had been achieved as of the filing date of the instant application. Thus, it is respectfully submitted that gene therapy, based on the availability of *in vivo*, animal and clinical data such as provided in these references, can not be considered an unpredictable field as of the filing date of the instant application.

Further, each of the articles cited in the Office Action as evidence of the alleged unpredictability of the art of gene therapy consistently identify the same problem areas in gene therapy (*i.e.*, gene delivery, sustained expression, immune reactions) and discuss specific means of confronting the problems and designing gene therapy protocols that have a greater likelihood of evoking a therapeutic response in the treated subject by avoiding as many of the problems as possible. The fact that limitations of gene therapy are so well-defined (as a result of extensive studies) attests to the predictability of the problems to be

encountered in practicing such methods and enables researchers to account for and to circumvent those problems that are most likely to be the greatest obstacle in any particular therapeutic program in their experimental programs. In fact, as discussed above, the instant application has addressed and overcome many of these problems.

Furthermore, it appears that the Office Action, in asserting the unpredictability of the art of gene therapy has equated "limitations" with "unpredictability." It is respectfully submitted that although certain methods of gene therapy can be associated with certain limitation and in fact, limited success, this does not establish the art as unpredictable. Furthermore, one of the major obstacles in the references cited by the Examiner is the inefficiency of gene delivery. The teachings of the instant application clearly provide remedies to the practitioner as the instant application is directed to methods of delivering nucleic acid molecules.

Furthermore, many of the allegations of unpredictability made by the Examiner are in reference to the lack of a particular level of clinical efficacy or optimization of gene therapy. As discussed above, as of the filing date of the instant application, the clinical efficacy of gene therapy had been demonstrated in several of the cited references as well as additional references provided by Applicant herein. The Applicant is not aware of any requirement under current U.S. patent law specifying particular minimum levels of optimization and certified efficacy in order for a treatment-related area of art to qualify as sufficiently "predictable" such that lack of enablement under 35 U.S.C. § 112, first paragraph, is not a consideration. The relevant standard is not that of an established, fully optimized, clinical course of treatment; rather, even in an *unpredictable* art, a patent application satisfies the requirements of 35 U.S.C. § 112, first paragraph, as long as it provides sufficient disclosure, either through illustrative examples or terminology, to teach those of ordinary skill how to make and use the claimed subject matter with reasonable, but not undue,

experimentation. There is no requirement that a treatment method achieve a specified level of efficacy or efficiency in order to be considered "enabled" by the specification.

As such, it is respectfully submitted, that although the art of gene therapy may not have been an optimized and perfected practice at the effective filing date of the subject application, the availability of clinical trial and animal data from the cited references establish that it was not so unpredictable as to qualify as a major factor in the determination of whether the requirements of 35 U.S.C. § 112, first paragraph, are satisfied with respect to the instantly claimed subject matter.

Conclusions

In light of the extensive teachings and examples in the specification for the delivery of nucleic acid molecules, the high level of skill of those in this art, the knowledge of those of skill in the art, the fact that it is predictable given the teachings of the instant application to deliver nucleic acid molecules for use in gene therapy applications and the breadth of the claims, it would not require undue experimentation for one of skill in the art to make and use the method of nucleic acid molecule delivery methods as taught by the instant application.

Accordingly, a consideration of the factors enumerated in Ex parte Forman leads to the conclusion that undue experimentation would not be required to introduce a nucleic acid molecule into a cell using the methods of the instant application, regardless of whether the delivery is *in vitro*, *in vivo* or *ex vivo* and regardless if the end-use is for gene therapy or for other purposes as taught by the instant application.

B. The written description rejection

I) Claims 1-26, 28-33, 38, 39, 41-48, 53-57, 65, 69, 71-76, 140 and 141 are rejected under 35 U.S.C. § 112, first paragraph because it is alleged that the specification does not describe the subject matter in such a way as to convey to one skilled in the relevant art that the inventor(s) had possession of the claimed

subject matter at the time the application was filed. In particular, it is alleged that "delivery agents", although defined in the specification, covers an enormous breadth of a genus that includes all compounds, conditions and physical treatments that enhance delivery of a nucleic acid into a cell and that the examples provided are limited to cationic compounds and two forms of energy, which are not representative of the full genus. It is further alleged that the specification does not limit the genus to a compound of a given structure, to any given form of energy or type of physical treatment. It is asserted that a delivery agent is a critical element of the claimed subject matter and that it is not sufficient to describe such an element solely by its principal biological property, *e.g.* enhancing contact of nucleic acid molecules with cells and/or increasing permeability of cells to nucleic acid molecules. Furthermore, it is asserted that naming a material or physical treatment generically thought to exist, in the absence of knowledge of such material or treatment, is inadequate to meet the written description requirement. In view of these factors, it is alleged that adequate written description is not provided commensurate with the scope of the claims.

This rejection is respectfully traversed. It is respectfully submitted that this rejection has been rendered moot with respect to Claim 140, which has been canceled.

Relevant Law

The purpose behind the written description requirement is to ensure that the patent applicant had possession of the claimed subject mater at the time of filing of the application In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). The manner in which the specification meets the requirement is not material; it may be met by either an express or an implicit disclosure.

35 U.S.C. §112 requires a written description of the invention. This requirement is distinct from and not coterminous with the enablement requirement:

> The purpose of the 'written description' requirement is broader than to merely explain how to 'make and use'; the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." Vas-Cath, Inc. v. Mahurkar, 935 F.2d at 1563-64, 19 USPQ2d at 1117 (emphasis in original).

The issue with respect to 35 U.S.C. §112, first paragraph, adequate written description has been stated as:

> [d]oes the specification convey clearly to those skilled in the art, to whom it is addressed, in any way, the information that appellants invented that specific compound [claimed embodiment] Vas-Cath, Inc. v. Mahurkar, at 1115, quoting In re Ruschig, 390 F.2d 1990, at 995-996, 154 USPQ 118 at 123 (CCPA 1967).

A specification must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, *i.e.*, whatever is now claimed. Vas-Cath, Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ.2d 1111, 1117 (Fed. Cir. 1991). A written description requirement issue generally involves the question of whether the subject matter of a claim is supported by or conforms to the disclosure of an application as filed. The test for sufficiency of support in a patent application is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." Ralston Purina Co. v. Far-Mar-Co., Inc., 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed. Cir. 1985) (quoting In re Kaslow, 707 F.2d 1366, 1375, 217 USPQ 1089, 1096 (Fed. Cir. 1983)) (see also, MPEP 2163.02).

An objective standard for determining compliance with the written description requirement is "does the description clearly allow persons of skill in the art to recognize that he or she invented what is claimed." In re Gosteli, 872 F.2d 1008, 1012, 10 USPQ.2d 1614, 1618 (Fed. Cir.1989).

The Examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in an applicant's disclosure a

description of the invention defined by the claims. In re Wertheim, 541 F.2d 257, 265, 191 USPQ 90, 98 (CCPA 1976); *See also* Ex parte Sorenson, 3 USPQ.2d 1462, 1463 (Bd. Pat.App. & Inter. 1987). By disclosing in a patent application a device that inherently performs a function or has a property, operates according to a theory or has an advantage, a patent application necessarily discloses that function, theory or advantage, even though it says nothing explicit concerning it. The application may later be amended to recite the function, theory or advantage without introducing prohibited new matter. In re Reynolds, 443 F.2d 384, 170 USPQ 94 (CCPA 1971); and In re Smythe, 480 F.2d 1376, 178 USPQ 279 (CCPA 1973).

Furthermore, the subject matter of the claims need not be described literally (*i.e.*, using the same terms or *inhaec verba*) in order for the disclosure to satisfy the description requirement. If a claim is amended to include subject matter, limitations, or terminology not present in the application as filed, involving a departure from, addition to, or deletion from the disclosure of the application as filed, the examiner should conclude that the claimed subject matter is not described in that application. This conclusion will result in the rejection of the claims affected under 35 U.S.C.112, first paragraph - description requirement, or denial of the benefit of the filing date of a previously filed application, as appropriate.

Analysis

1) The Examiner alleges that a delivery agent is a critical element of the claimed subject matter and as such is not sufficiently described.

Applicant respectfully submits that the claims are directed to methods of nucleic acid delivery, not to delivery agents *per se*. Further, as discussed below, the delivery agents used in the instantly claimed methods are adequately described in the specification and were also well known and structurally and functionally characterized at the time the instant application was filed.

As discussed above with respect to the enablement rejection, the specification describes each of the steps of the claimed methods for introducing a nucleic acid molecule into a cell by exposing the nucleic acid to a delivery agent, exposing the cell to a delivery agent, and contacting the cell with the nucleic acid molecule. Further, the specification describes the introduction of a nucleic acid molecule *in vitro*, *in vivo* and *ex vivo* as well as the introduction of a nucleic molecule into a cell of a subject that can be used for gene therapy as well as other applications.

As further discussed above with respect to the enablement rejection, the specification provides working examples of specific embodiments that encompass these steps such as: introduction of a nucleic acid molecule using specific ordered steps; the use of energy and cationic compounds as delivery agents; the use of combinations of delivery agents; introduction of large nucleic acid molecules; and the introduction of nucleic acids into a variety of cell types. The specification also describes methods of labeling, detecting labeled nucleic acid molecules and measuring the delivery of nucleic acid molecules. Additionally, methods of gene expression and detecting gene expression from nucleic acid molecules delivered to cells are described. Thus, each step of the claimed methods, which are directed to delivering a nucleic acid molecule to a cell using delivery agents, are described in the specification in a manner demonstrating that the Applicant had possession of the claimed subject matter at the time of filing of the application.

2) The Office Action alleges that the examples provided by the instant application describe only cationic compounds and two forms of energy and that this is insufficient to cover the breadth of the genus (of delivery agents) as claimed. The Office Action, citing Regents of the Univ. Calif. v. Eli Lily & Co., 43 USPQ2d 1398 (CA FC, 1997) and Fiers v. Revel, 25 USPQ2d 1601 (CA FC 1993), further alleges that claiming methods that achieve a certain result

without defining what means will do so is not in compliance with the written description requirement.

It is respectfully pointed out to the Examiner that many classes of delivery agents are described in the specification. For example, at page 19, lines 12-14, the specification defines delivery agents to include agents such as peptides, proteins, and cavitation compounds in addition to cationic compounds and energy. Further, at page 19, lines 20-23, additional species of delivery agents are provided, for example compounds and chemical compositions such as calcium phosphate, DMSO, glycerol, chloroquine, sodium butyrate, polybrene, DEAE-dextran, peptides, proteins, and physical treatments such as temperature, light, pH, radiation and pressure. This description represents numerous diverse species in addition to cationic compounds, ultrasound and electroporation that are representative of a genus of delivery agents as instantly claimed.

Further, Applicant respectfully disagrees with the Examiner's comparison of the instant claims to those of Regents of the Univ. Calif. v. Eli Lilly & Co., 43 USPQ2d 1398 (CA FC, 1997) and Fiers v. Revel, 25 USPQ2d 1601 (CA FC 1993) for alleged lack of written description. Unlike the claims at issue in the cited cases, the instant claims are method claims, not composition of matter claims. Moreover, the claims in the cited cases were directed to nucleic acid molecules whose sequences had yet to actually be discovered or physically isolated and sequenced. The delivery agents used in the methods of the instant application, on the other hand, have been described in great detail in the specification and were known to those of skill in the art at the time the instant application was filed (*see* discussion above and below). As described in detail above and below, delivery agents were readily available, conventional reagents and treatments recognized, available, and known to those of skill in the art at the timing of filing of the instant application.

3) The Examiner further alleges that the term "delivery agent" is naming a material or physical treatment generically thought to exist, in the absence of knowledge of such material or treatment.

Applicant respectfully asserts that delivery agents are well-known reagents that were available to one skilled in the art at the timing of filing of the instant application. These reagents were commercially available at the time of filing and are listed (with details of availability) in the specification. For example, the cationic and non-cationic compounds described at pages 20-21 are listed as being available from such manufacturers of biotechnology reagents listed therein such as Life Technologies, Qiagen, Promega, Biontex, Clontech and Gene Therapy Systems.

Additionally, delivery agents such as those described in the instant application were known to those of skill in the art at the time the instant application was filed. The instant application cites numerous patents, published patent applications and scientific journal publications for delivery agents, such as LipofectAMINE (page 20, line 29), LipofectAMINE PLUS (page 21 lines 2-4) LipofectAMINE 2000 (page 21, lines 4-5), SAINT-2 (page 21, lines 28-31) and calcium phosphate (page 41, lines 2-4). Publications and patents are also cited for electroporation (page 23, lines 24-25) and ultrasound (page 22, line 31 through page 23, line 2). Furthermore, many delivery agents were available at the time of filing of the instant application from standard chemical companies well known by one skilled in the art, such as Sigma. For example, delivery agents such as calcium phosphate, DMSO, glycerol, chloroquine, sodium butyrate, polybrene, DEAE-dextran (page 19, lines 20-22) are each listed in the 1998 Sigma catalog (copies of relevant pages are provided in a Supplemental Information Disclosure statement filed on the same day herewith). Further, delivery agents such as light, radiation, pH and temperature are all reagents known to one of skill in the art at the time the instant application was filed, commonly available and routinely used by one skilled in the art of DNA delivery.

The conventional knowledge of delivery agents in the art is evidenced by publications of Luo *et al.*, Uherek *et al.*, and Han *et al.* (provided in a Supplemental Information Disclosure statement filed on the same day herewith), which pre-date the instant application. For example, Luo *et al.* (2000) *Nature Biotechnology 18*:33-37 reviews DNA delivery systems. This reference cites numerous delivery agents known and in use in the art such as pressure, particle bombardment, high and low voltage electroporation, DEAE-dextran, calcium phosphate, polymers and encapsulated polymers, and proteins and peptides such as low density lipoprotein, Gal4-invasin, polylysine and protamine sulfate (see, for example, Table 1, page 34 and Figure 2, page 35). Peptide gene delivery systems are reviewed by Uherek *et al.* (2000) *Adv. Drug Delivery Reviews 44*:153-166, including viral proteins, receptors, protein fusions with DNA binding proteins and toxin translocator fusions used in DNA delivery. Han *et al.* (2000) *Molec. Therapy 2*:302-317 describes a multitude of biomaterials for gene therapy. Han *et al.* describes cationic liposomes, polyamines, such as polyethyleneimine (PEI), polylysine (PLL) and polyamidoamide (PANAM) and conjugates to polyamines such as transferrin-PLL and folate-PLL (page 303, col. 1 ¶¶ 2-4). Han *et al.* further describes other delivery agents in the art such as polymers, for example chitosan, dendrimers, polyvinyl alcohol, polyvinylpyrrolidone, and poly [*α*-(4-aminobutyl)-L-glycolic acid] (PAGA) (pages 305-307). The use of temperature with polymers for DNA delivery is also described (pages 308-309). Thus, as evidenced by these publications and the commercial availability of delivery agents, delivery agents were well known in the art at the time of filing of the instant application. Representative species across the broad genus such as cationic and non-cationic agents, energy such as electrical energy and temperature, peptides, proteins and polymers were all known to one of skill in the art.

It is not necessary to include in the specification that which those of skill in the art know. The specification is presumed to include all such knowledge.

As discussed above, delivery agents for use in the methods as instantly claimed were well- recognized and understood by those of skill in the art at the time the instant application was filed. These delivery agents were recognized by their physical and structural properties and not merely by their functional ability to mediate nucleic acid delivery. As demonstrated by the above exemplary publications, knowledge of such delivery agents and, thus, it is not necessary to describe such agents in detail in the specification. The failure to do so, certainly does not evidence that the inventors did not appreciate their discovery at the time of filing of this application. Nonetheless, as also discussed above and below, Applicant has in fact provided extensive descriptions of a variety of these known delivery agents. Thus, the recitation of "delivery agent" and the description of examples of classes of delivery agents representative of the genus, when coupled with the knowledge in the art of the types of delivery agents as discussed above, is sufficient to define the genus and provide adequate written description for "delivery agent" for use in the nucleic acid delivery methods as claimed.

Therefore, Applicant had possession of the claimed subject matter at the time of filing of the application.

4) The Examiner contends that it is not sufficient to define delivery agent solely by its principal biological property.

In response, it is noted that in the instant application, it is not the delivery agents that are being claimed, but methods of nucleic acid delivery using delivery agents that were well-known to those of skill in the art at the time the instant application was filed. Further, recognition of molecules as delivery agents by their physical or structural characteristics rather than their functional property (use in delivery) was well established in the art at the time the instant application was filed and have been identified as such in the specification. For example, as described by Luo *et al.* (*supra*), delivery agents fall into three broad classes: mechanical, such as pressure and particle bombardment; electrical,

such as electroporation; and chemical, such as cationic lipids, calcium phosphate and proteins (see for example, Table 1, page 34 and the discussion of each class pp. 33-35). Each of these classes has common structural features known to one skilled in the art and thus are recognized by one skilled in the art as delivery agents for use in the methods of the instant application. Representative species of each of these broad classes of delivery agents, mechanical, electrical and chemical, are described in the instant application and their physical and structural properties were well-known to those of skill in the art at the time the instant application was filed. For example, cationic compounds such as cationic lipids and non-lipids are described in detail on pages 13-14 and pages 20-24. Additional chemical and biochemical delivery agents of known structure are disclosed on page 19 (lines 20-22). The specification discloses several species of energy and treatments. For example, the specification discloses types of ultrasound and ultrasound treatments on page 14 and on pages 22-23. Electroporation and treatments using electroporation are described on page 23, lines 22-26. Additional types of energy such as radiation and light and additional types of treatments such as pH, temperature, and mechanical treatments, such as pressure, are disclosed on page 19, lines 22-23. Thus, it is clear from the instant application that the Applicant was in possession of the genus of delivery agents as claimed at the time the application was filed.

Further, an adequate written description for a claimed genus need only provide "relevant, identifying characteristics" of a representative number of species (MPEP §2163). The instant specification clearly describes structurally and functionally known delivery agents that are known to mediate their effects through interactions with nucleic acid molecules and/or cell membranes to deliver a nucleic acid molecule to a cell. The instant application teaches one to deliver a nucleic acid molecule using delivery agents that are readily identified by

their physical and structural characteristics as described in the application and as known to those of skill in the art at the time the instant application was filed.

Applicant is entitled to claims that are commensurate in scope not only with what applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed. As discussed above, Applicant discloses and describes to the public methods with numerous types of delivery agents representative of the genus. Additionally, many such delivery agents are described and characterized in great detail in the instant application (see for example, Example 4, discloses the use of Superfect to deliver large nucleic acid molecules, Example 5 discloses the use of ultrasound, Example 6 discloses the use of ultrasound with Saint-2 and Example 7 discloses numerous delivery agents such as Clonfectin, Cytofectin, Effectene, Eufectins 1 through 11, fugene 6, geneporter 2, lipofectamine, lipofectamine 2000, metafectene and superfect).

To require Applicant to limit the claims to specific species of the broad class of delivery agents would permit those of skill in the art to practice what is described in the specification but avoid infringing claims so-limited. If Applicant is required to limit the claims to only the aforementioned delivery agents, then those of skill in the art could by virtue of the teachings of this application readily practice what is claimed by substituting another delivery agent and practice what is disclosed in the application, but avoid infringing such limited claims. To permit that is simply not fair. The instant application describes methods of delivering a nucleic acid molecule to a cell using a broad range of delivery agents. Having done so, it is now routine for others to deliver a nucleic acid molecule to a cell using these methods. Those of skill in the art should not be permitted to make such minor modifications by substitution of a different delivery agent and avoid infringing such claims.

II. **Claims 31 and 33** are additionally rejected under 35 U.S.C. § 112, first paragraph for lack of written description because it is alleged that the

specification is lacking adequate written description for " a cell capable of the generation of a specific organ." This rejection is respectfully traversed.

Relevant law

See above.

Analysis

Claims 31 and 33 are directed to the method of Claim 1 for delivery of a large nucleic acid molecule into a cell in which the cell is selected from the group consisting of a stem cell, a primary cell, a cell from an immortalized cell line, a nuclear transfer cell, an embryonic cell and a cell capable of the generation of a specific organ.

The Office Action alleges that the specification does not provide a single example of a cell encompassed by the genus of "cells capable of the generation of a specific organ". Applicant respectfully submits that the specification provides several examples of a cell capable of generating a specific organ. For example, on page 6, lines 3-9, the specification describes methods of delivering large nucleic acid molecules into a cell for the production of transplantable organs. Such cells include embryonic stem cells, stem cells and a nuclear transfer donor cell. As discussed below, these cells are known in the art to be capable of generating organs such as liver, skin, kidney, gut, neurons, epidermis, and bone. At page 26, lines 1-5, the specification describes organs that are targets for *in vivo* gene therapy, for example, skin, stomach, intestine, lung, bladder, uterus, liver, kidney, pancreas, brain, heart, spleen, prostate and joints (*e.g.*, the knee, elbow, shoulder, wrist, hip). It is known in the art that cells of these organs are capable of forming such organs. Further, as discussed above, the specification describes in detail and provides many examples of the delivery of DNA to such cell types.

As discussed above, it is not necessary to include in the specification that which those of skill in the art know. The specification is presumed to include all such knowledge. Cells capable of generation of organs were well-

recognized and understood by those of skill in the art at the time the instant application was filed. To evidence the state of the art regarding "cells capable of the generation of an organ," Applicant provides the publications of Tsonis (2000) *Devel. Biol. 221*:273-284, Stocum (1999) *Cell and Devel. Biol. 10*:433-440 and Stocum (1998) *Wound Repair and Regeneration 6*:276-290 (provided in a Supplemental Information Disclosure statement being filed on the same day herewith).

Tsonis reviews regeneration in vertebrates including the ability of cells to form complex organs. Tsonis describes many cell types that are capable of organ generation (see especially Table 1, page 274) such as simple cell proliferation in intestines, liver and adrenal glands for regeneration of those organs. Additionally, proliferation and differentiation of stem cells regenerates other types of organs (page 273, col. 2, ¶2). Also described by Tsonis are cells capable of dedifferentiation and transgeneration that produce an exact replica of a lost part such as in limb and eye formation, *e.g.*, in amphibians (page 275, col. 1 ¶1). Organs are created and recreated through these regenerative processes.

Further evidence of knowledge in the art regarding cells capable of generating an organ is found in Stocum (1999). This reference reviews the art of regenerative biology including the differentiation of cells into organs and the formation of bioartificial organs from cells. Stocum (1999) describes the capability of cell types to regenerate organs. For example, stem cells are capable of forming muscle, liver and pancreas. Further described are embryonic stem cells with the capability of forming virtually every tissue in the body and are thus useful for formation of many organ types. For example, when injected into SCID mice, ES cells formed kidney, gut, neurons, epidermis, muscle and bone (page 435, col. 2, ¶1). Also known in the art at the time of filing were many cell types capable of generating, *e.g.*, bioartificial organs for transplantation. The article of Stocum (1998) reviews strategies for

regeneration including organ formation from different cell types. Stocum (1998) describes the use of fibroblasts, stem cells, and pancreatic cells to generate organs such as bioartificial skin, pancreas, bone and cartilage (see, for example, page 279).

Thus, the recitation of "a cell capable of generation of a specific organ" coupled with the knowledge in the art that many types of cells were known to be capable of generating organs and the examples within the instant application of such cell types is sufficient to define the genus and provide adequate written description for "a cell capable of the generation of an organ" for use in the nucleic acid delivery methods as claimed.

As demonstrated by the above exemplary publications, cells capable of generation of organs were known to those of skill in the art at the time the instant application was filed and, thus, it is not necessary to describe such cells in detail in the specification. The failure to do so, certainly does not evidence that the inventors did not appreciate their discovery at the time of filing of this application.

Therefore, Applicant had possession of the claimed subject matter at the time of filing of the application.

REJECTION OF CLAIM 50 UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claim 50 is rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to point out and distinctly claim the subject matter. Specifically the Office Action alleges that "the cationic compound" has no antecedent basis in claim 48 on which the claim depends.

Claim 50 is amended to more particularly point out the claimed subject matter. In accordance with the Examiners suggestion, Claim 50 is amended to depend on Claim 49, which provides antecedent basis for "the cationic compound".

REJECTION OF CLAIMS 1, 7, 9, 10, 12-14, 30-32, 58, and 61-64 UNDER 35 U.S.C. § 102(b)

A. Claims 1, 7, 9, 10, 12-14, 30-32, 58, and 61-64 are rejected under 35 U.S.C. § 102(b) as being anticipated by Strauss *et al.* (*EMBO J.* (1992) 11: 417-422). The Office Action alleges that Strauss *et al.* anticipates claim 1 because it discloses a method of introducing a nucleic acid molecule into a cell that includes (a) exposing the nucleic acid molecule to a delivery agent (b) exposing the cell to a delivery agent; and (c) contacting the cell with the nucleic acid molecule where step (a) is performed first and steps (b) and (c) are performed simultaneously. Further, the Office Action alleges that Strauss *et al.* anticipates dependent claims 7, 9, 10, 12-14 and 30-32 because it discloses that the nucleic acid is an artificial chromosome, that it is exposed to the delivery agent *in vitro*, that the contact between the nucleic acid and the cell is effected *in vitro* , that the delivery agent comprises DOTMA and the cell is a primary animal cell.

The Office Action alleges that Strauss *et al.* anticipates claim 58 because Strauss discloses a method that includes contacting a nucleic acid molecule with a cationic lipid and contacting the nucleic acid molecule with a cell. It is also alleged that Strauss *et al.* discloses the limitations of claims 61-64, namely, that the nucleic acid molecule is an artificial chromosome, the cell is an animal cell and a primary cell and that the nucleic acid molecule is contacted with the cell *in vitro*.

Reconsideration of this rejection is respectfully requested in light of the amendments herein and the following remarks. It is respectfully submitted that this rejection has been rendered moot with respect to Claim 58, which is canceled herein. Furthermore, claims 61-64 are amended herein to depend from claim 59, which is not rejected on this basis. Therefore, claims 61-64 are outside the purview of this rejection.

Relevant law

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. In re Spada, 15 USPQ2d 1655 (Fed. Cir, 1990), In re Bond, 15 USPQ 1566 (Fed. Cir. 1990), Soundscriber Corp. v. U.S., 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, Richardson v. Suzuki Motor Co., 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention." In re Lang, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). Moreover it is incumbent on Examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference. Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co., 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984). Further, the reference must describe the invention as claimed sufficiently to have placed a person of ordinary skill in the art in possession of the invention. An inherent property has to flow naturally from what is taught in a reference In re Oelrich, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981).

"Rejections under 35 U.S.C. §102 are proper only when the claimed subject matter *is* identically disclosed or described in the "'prior art'" . . .the [r]eference must clearly and unequivocally disclose the claimed compound or direct those skilled in the art to the compound without *any* need for picking, choosing, and combining various disclosures not directly related to each other by the teachings in the cited references. Such picking and choosing may be entirely proper when making a rejection of a 103, obviousness rejection, where the applicant must be afforded an opportunity to rebut with objective evidence any inference of obviousness which may arise from the *similarity* of the subject matter which he claims to the prior art, but it has no place in the making of a 102, anticipation rejection." (Emphasis in original). In re Arkey, Eardly, and Long, 455 F.2d 586, 172 USPQ 524 (CCPA, 1972).

The claims

Independent Claim 1 is directed to a method of introducing a large nucleic acid molecule into a cell by exposing the cell to a delivery agent, exposing a large nucleic acid molecule to a delivery agent, and contacting the cell with the nucleic acid molecule. The steps can be performed in any order sequentially except if the delivery agent is energy it is not applied to the nucleic acid molecule or to the cell after contacting the cell with the nucleic acid molecule. Dependent claims further specify that the nucleic acid molecule is an artificial chromosome (claim 7); methods of exposing the nucleic acid molecule to the delivery agent or the cells to the nucleic acid *in vitro*, *ex vivo* and *in vivo* (claims 9 and 10); cationic compounds as delivery agents (claims 12-14); and cell types (claims 30-32).

Analysis

Differences between the claims and the disclosure of Strauss *et al.* (1992) *EMBO J.* 11:417-422.

Strauss *et al.* discloses methods for transfecting a 150 kilobase yeast artificial chromosome (YAC) into the fibroblast cell line M13-5. Strauss *et al.* incubates the YAC DNA with the lipid DOTMA (commercially known as Lipofectin) to form lipid-DNA micelles *in vitro* and then pours the mixture over a monolayer of M13-5 cells, exposing the cells to both the YAC and the DOTMA agent.

Strauss *et al.* does not disclose any method in which a large nucleic acid molecule is introduced into a cell. All of the rejected claims are directed to methods for the introduction of large nucleic acid molecules into cells, in which the large nucleic acid molecule and the cell are treated with delivery agent(s) and then the large nucleic acid molecule is contacted with the cell. As defined in the instant specification, *e.g.*, at page 9, line 30 to page 10, line 3, a large nucleic acid molecule refers to a nucleic acid molecule that is at least about 0.5 megabases (*i.e.*, 500 kilobases) in length. As stated above, the nucleic acid

molecule disclosed in Strauss *et al.* is at least several fold smaller than the nucleic acid molecules used in the delivery methods of the rejected claims. Strauss *et al.* does not disclose any methods for introducing an artificial chromosome of such size into any cell type or with any delivery agent. Further, Strauss *et al.* does not disclose sequentially exposing the nucleic acid and the cell to a delivery agent. As discussed above, in Strauss *et al.*, the cells are exposed simultaneously to the nucleic acid and the delivery agent.

All of the rejected claims (Claim 1 and claims dependent thereon) are directed to methods of introducing a large nucleic acid molecule (defined in the specification as at least about 0.5 megabases) into a cell, which include sequential steps of exposing a delivery agent to the cell, exposing a delivery agent to the nucleic acid and contacting the cell with the nucleic acid molecule. None of these elements of the rejected claims are disclosed in Strauss *et al.*

Therefore, since anticipation requires that a reference disclose all elements as claimed, Strauss *et al.*, which does not disclose introducing a large nucleic acid molecule into a cell, nor sequential exposure of the large nucleic acid and the cell to a delivery agent and contacting the nucleic acid with the cell, does not anticipate any of the Claims 1, 7, 9, 10, 12-14 and 30-32, all of which are directed to methods in which a large nucleic acid molecule is introduced into a cell and/or sequential exposure of the nucleic acid and the cell to a delivery agent.

B. Claims 1, 2, 9-17, 26-28, 30-32, 58, 61-64, 140, 142 and 143 are rejected under 35 U.S.C. § 102(b) as being anticipated by Unger *et al.* (*Invest. Radiol.* (1997) 32:723-727). The Office Action alleges that Unger *et al.* discloses a method of introducing a nucleic acid molecule into a cell that includes (a) exposing the nucleic acid molecule to a delivery agent (b) exposing the cell to a delivery agent; and (c) contacting the cell with the nucleic acid molecule where in step (a) the nucleic acid is exposed to a cationic compound and in step (b) the cells are exposed to ultrasound.

Reconsideration of this rejection is respectfully requested in light of the amendments herein and the following remarks. It is respectfully submitted that this rejection has been rendered moot with respect to Claims 58 and 140, which are canceled herein. Furthermore, claims 61-64 are amended herein to depend from claim 59, which is not rejected on this basis. Therefore, claims 61-64 are outside the purview of this rejection.

Relevant law

See above.

The claims

Independent Claim 1 is as described above. Dependent claims are as described above and additionally claims 2, 11, 15-17, 19, and 26-28 specify particulars such as agents that increase contact between the nucleic acid molecule and the cell and agents that enhance cell permeability (claim 2); and types of delivery agents, treatments using the delivery agents, and the order in which the steps of the methods are practiced (claims 11, 15-17, 19, and 26-28). Claims 142 and 143 specify types of delivery agents in the method of Claim 141, which is directed to a kit for delivering nucleic acids into cells that contains a composition comprising an artificial chromosome, a delivery agent that includes a composition comprising a delivery agent, reagents for performing sonoporation or electroporation and optionally instructions for delivering nucleic acids into cells.

Analysis

Differences between the claims and the disclosure of Unger *et al.* (*Invest. Radiol.* (1997) 32:723-727)

Unger *et al.* discloses a method for transfecting plasmids into mammalian cells by applying a complex of plasmid DNA and liposomes to cells. Ultrasound energy is applied to the cells after adding the liposome-DNA complex to enhance gene expression from a reporter gene encoded by the plasmid. Unger *et al.* does not disclose any methods for transfecting large nucleic acid molecules as

disclosed and claimed herein. As discussed above, the rejected claims are directed to methods for the introduction of large nucleic acid molecules into cells, where the large nucleic acid molecules that are contacted with the cells are defined in the specification as being at least 0.5 megabases.

Unger *et al.* discloses transfection of the plasmid pCMVCAT using liposomes containing the cationic compounds DPEPC and DOPE or DMRIE. Unger *et al.* discloses the transfection of this plasmid into three mammalian cell lines, human epitheloid carcinoma, mouse mammary tumor, and mouse embryo. The plasmid, pCMVCAT, of Unger *et al.* was obtained from Life Technologies, Inc. (see page 24, first full paragraph) and has a size of 5 kilobases (*see* Example 9, col. 10, lines 20-22 of U.S. Patent No. 5,334,761 (a copy is provided in a Supplemental Information Disclosure statement being filed on the same day herewith), granted to Life Technologies, Inc.). Thus, the nucleic acid molecule used in the delivery methods of Unger *et al.* is at least about a 100-fold smaller than the nucleic acid molecules of at least about 0.5 megabases that constitute the "large" nucleic acid molecules of the instantly claimed methods. Further, in contrast to claim 1 and dependent claims 2, 9-17, 19, 26-28, 30-32, Unger *et al.* discloses that the optimal time for applying ultrasound energy to the cells is <u>after</u> the application of the DNA-liposome complex to the cells (*see, e.g.*, Figure 4 and page 725, col. 1, para. 2). The rejected claims specify that if the delivery agent is energy, it is <u>not</u> applied to the cell <u>after</u> contacting the cell with the nucleic acid molecule.

Unger *et al.* does not disclose any method of introduction of a large nucleic acid molecule of (defined as at least about 0.5 megabases) into cells. Therefore, since anticipation requires that a reference disclose all elements of the methods as claimed, Unger *et al.*, which does not disclose a method of introducing a large nucleic acid molecule of at least about 0.5 megabases into a cell, does not anticipate any of the claims 1, 7, 9, 10, 12-14 and 30-32. Further, with respect to Claims 142 and 142, Unger *et al.* does not disclose any

methods for delivery of artificial chromosomes, nor kits containing artificial chromosomes. Therefore, Unger *et al.* does not anticipate Claims 142 and 143.

C. Claims 1, 9, 10, 12-14, 30-32, 58, 61-66, 70 and 72 are rejected under 35 U.S.C. § 102(b) as being anticipated by McDonald *et al.* (US 5,837,283). The Office Action alleges that McDonald *et al.* discloses a method of introducing a nucleic acid molecule into a cell that includes (a) exposing the nucleic acid molecule to a delivery agent (b) exposing the cell to a delivery agent; and (c) contacting the cell with the nucleic acid molecule where step (a) is performed first and steps (b) and (c) are performed simultaneously. Reconsideration of this rejection is respectfully requested in view of the amendments herein and the following remarks. It is respectfully submitted that this rejection has been rendered moot with respect to Claim 58, which is canceled herein. Furthermore, claims 61-64 are amended herein to depend from claim 59, which is not rejected on this basis. Therefore, claims 61-64 are outside the purview of this rejection.

Relevant law

See above.

The claims

Claims 1, 9, 10, 12-14, 30-32, 58, 61-64 are as described above. Claim 65 is directed to a method of delivering a nucleic acid molecule into a cell of a subject that includes mixing the nucleic acid molecule with a delivery agent; administering the mixture to the subject and applying ultrasound or electrical energy to the subject where the combination provides a greater extent of delivery of the nucleic acid than using either the agent or energy alone. Dependent claims further specify types of delivery agents and types of nucleic acid molecules for use with the method.

Analysis

Differences between the claims and the disclosure of McDonald *et al.* (US 5,837,283)

McDonald *et al.* is directed to a method of specifically targeting angiogenic endothelial cells by delivering lipid/DNA complexes or cationic liposomes containing a substance that affects the targeted cells by inhibiting or promoting their growth. The cationic liposome compositions used in the methods of McDonald *et al.* have a greater affinity for angiogenic endothelial cells than normal endothelial cells.

In one embodiment, DNA is delivered to angiogenic endothelial cells using cationic lipid/DNA complexes. The DNA is mixed with liposomes and injected into a mouse.

McDonald *et al.* does not disclose the introduction of a large nucleic acid molecule into cells. As discussed above, the large nucleic acid molecules delivered according to the methods of instant claim 1 and dependents are defined as being at least 0.5 megabases in size. McDonald *et al.* only discloses the delivery of plasmid DNA and small promoter-gene constructs of the order of no more than a few kilobases (about 5-6 kilobases or under; *see* col. 12, lines 45-65 and col. 20, line 33 to col. 21, line 54, including WO 93/12240, incorporated by reference at col. 20, lines 42-43 for specific disclosure and description of the formation of nucleotide sequence/lipid complexes) into cells. The sizes of the DNA molecules disclosed in McDonald *et al.* are at least two orders of magnitude smaller than the large nucleic acid molecules used in the instantly claimed methods of, *e.g.*, claim 1 and dependents. Further, unlike instant claim 1 and dependents, McDonald *et al.* does not disclose sequential exposure (in any order) of the nucleic acid to a delivery agent, the cell to a delivery agent, and the nucleic acid to the cell.

The disclosure of McDonald *et al.* also focuses on delivery of liposomes, with or without DNA, to angiogenic endothelial cells. McDonald *et al.*

McDonald *et al.* does not provide any general methods for the delivery of nucleic acids, much less large nucleic acids, to cells. McDonald *et al.* also does not disclose the use of energy as a delivery agent for nucleic acids or for any other substances.

In contrast to Claim 1 and dependent Claims 9, 10, 12-14 and 30-32, McDonald *et al.* does not disclose the introduction of a large nucleic acid molecule (defined as at least about 0.5 megabases) into cells. Further, in contrast to Claim 65 and dependent Claims 66, 70 and 72, McDonald *et al.* does not disclose the use of energy in addition to another delivery agent so that the nucleic acid molecules are delivered to the cells to a greater extent than when either the delivery agent or energy alone is used.

Therefore, since anticipation requires that a reference disclose all elements as claimed, McDonald *et al.*, which does not disclose introducing large nucleic acid molecules into a cell, does not anticipate any of the claims 1, 9, 10, 12-14 and 30-32. Further, McDonald *et al.* does not anticipate any of claims 65, 66, 70 and 72, all of which specify use of a delivery agent mixed with a nucleic acid molecule and the application of energy so that the nucleic acid molecule is delivered into the cell to a greater extent than using the delivery agent or energy alone.

D. Claims 1, 3-10, 12-14, 30-33, 58, and 60-64 are rejected under 35 U.S.C. § 102(b) as being anticipated by Hadlaczky *et al.* (US 6,025,155) because Hadlaczky *et al.* allegedly discloses a method of introducing an artificial chromosome into a cell with lipid-mediated transfer that one of ordinary skill in the art would understand to include (a) exposing the nucleic acid molecule to a delivery agent (b) exposing the cell to a delivery agent; and (c) contacting the cell with the nucleic acid molecule where step (a) is performed first and steps (b) and (c) are performed simultaneously. Reconsideration of this rejection is respectfully requested in view of the amendments herein and the following remarks. It is respectfully submitted that this rejection has been rendered moot

with respect to Claims 58 and 60, which are canceled herein. Furthermore, claims 61-64 are amended herein to depend from claim 59, which is not rejected on this basis. Therefore, claims 61-64 are outside the purview of this rejection.

Relevant law

See above

The claims

Claims 1, 7, 9, 10, 12-14 and 30-32 are described above. As noted above, claim 1 is directed to a method for delivery of nucleic acid to a cell that includes the steps of:

(a) exposing a large nucleic acid molecule to a delivery agent;

(b) exposing the cell to a delivery agent; and

(c) contacting the cell with the nucleic acid molecule. The steps are performed sequentially in any order, provided that if the delivery agent is energy it is not applied to the nucleic acid molecule and it is not applied to the cell after contacting the cell with the nucleic acid molecule. Claims 6-8 specify types of nucleic acid molecules such as an artificial chromosome or ACes used in the method of Claim 1. Claim 33 further specifies cell types for use with the method of Claim 1.

Analysis

Differences between the claims and the disclosure of Hadlaczky *et al.* (US 6,025,155)

Hadlaczky *et al.* discloses methods for making and using artificial chromosomes. Hadlaczky *et al.* discloses that artificial chromosomes can be introduced into cells by a variety of methods. Hadlaczky *et al.* does not disclose methods that include sequential exposure of a nucleic acid to a delivery agent, a cell to a delivery agent and the nucleic acid to the cell.

Claims 1, 3-10, 12-14 and 30-33 are each directed to methods of introducing a large nucleic acid molecule into a cell that include specific

sequential steps. For example, independent Claim 1 specifies the steps of: a) exposing the cell to a delivery agent, b) exposing the nucleic acid molecule to a delivery agent, and c) contacting the cell with the nucleic acid molecule; with further specifications as to the sequential order of the steps and particular conditions when the delivery agent is energy.

The Examiner alleges that one of ordinary skill in the art would understand the disclosure of Hadlaczky *et al.* to include a method containing the steps of (a) exposing the nucleic acid molecule to a delivery agent (b) exposing the cell to a delivery agent; and (c) contacting the cell with the nucleic acid molecule. The Examiner points to the paragraph bridging columns 5 and 6, Example 1 and the text beginning at column 24 to support this allegation.

The paragraph bridging columns 5 and 6 lists known reagents and treatments for introducing DNA such as calcium phosphate, electroporation and lipid-mediated transfer. This paragraph does not disclose a method employing such reagents for the delivery of large nucleic acids such as artificial chromosomes, that include the steps of contacting the nucleic acid molecule with a delivery agent, contacting the cell with a delivery agent and contacting the cell with the nucleic acid, where the steps are performed sequentially in any order.

Further, the text at column 24 (and through column 27) addresses specific uses for mammalian artificial chromosomes. It is described that exogenous DNA such as a CFTR gene or genomic DNA library fragments can be incorporated into artificial chromosomes and then introduced into cells. This text does not disclose the steps of the methods of the instant application for introducing large nucleic acid molecules such as artificial chromosomes.

Furthermore, Example 1 (starting at column 28) discloses that plasmid DNA can be introduced into cell lines already containing artificial chromosomes (see A.3. at lines 24-31 of column 28). Example 1 also describes that artificial chromosomes can be transferred to recipient cell types using microcell-mediated

transfer in which donor and recipient cells are fused and hybrid cells are produced (see A.4. and A.5. beginning at line 32 of column 28).

None of the noted passages and/or the Example describe a method including the steps of exposing the nucleic acid molecule to a delivery agent; exposing the cell to a delivery agent; and contacting the cell with the nucleic acid molecule performed sequentially in any order.

For instance, Hadlaczky *et al.* does not disclose that a nucleic acid is exposed to a delivery agent, that a cell is exposed to a delivery agent or that the cell is contacted with the nucleic acid molecule sequentially in any order except for a particular order when the delivery agent is energy. Hadlaczky *et al.* does not disclose that when energy is used as a delivery agent, it is not applied to the nucleic acid molecule nor to the cell after contacting the cell with the nucleic acid molecule.

Therefore, since anticipation requires that a reference disclose all elements as claimed, Hadlaczky *et al.*, which does not disclose any method of introducing a large nucleic acid molecule into a cell that includes the steps of (a) exposing a large nucleic acid molecule to a delivery agent (b) exposing the cell to a delivery agent; and (c) contacting the cell with the nucleic acid sequentially in any order except when energy is used as a delivery agent, it is not applied to the nucleic acid molecule nor to the cell after contacting the cell with the nucleic acid molecule, does not anticipate any of the claims 1, 6-10, 12-14 and 30-33, nor any pending claims.

* * *

In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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